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NATIONAL INSTITUTE OF ARTHRITIS AND  
MUSCULOSKELETAL AND SKIN DISEASES

ANNUAL REPORTS

INTRAMURAL RESEARCH PROGRAMS

OCTOBER 1, 1993 TO SEPTEMBER 30, 1994  
(6040 FORMS ONLY)



925  
N277  
1994

PROJECT NUMBERS  
NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Z01 AR 27000-32 LPB

Z01 AR 41086-05 LSB

Z01 AR 27001-20 LPB

Z01 AR 41087-05 LSB

Z01 AR 27003-35 LPB

Z01 AR 41089-03 LSB

Z01 AR 27004-25 LPB

Z01 AR 41090-03 LSB

Z01 AR 27012-10 LPB

Z01 AR 41091-03 LSB

Z01 AR 27014-03 LPB

Z01 AR 41093-03 LSB

Z01 AR 27002-16 LSBR

Z01 AR 27013-03 LSBR

TERMINATED PROJECTS

Z01 AR 41025-23 ARB

Z01 AR 27005-12 LPB

Z01 AR 41048-15 ARB

Z01 AR 41022-22 ARB

Z01 AR 41066-12 ARB

Z01 AR 41023-20 ARB

Z01 AR 41074-07 ARB

Z01 AR 41040-22 ARB

Z01 AR 41076-07 ARB

Z01 AR 41097-03 ARB

Z01 AR 41083-05 ARB

Z01 AR 41098-03 ARB

Z01 AR 41088-04 ARB

Z01 AR 41092-03 ARB

INACTIVE PROJECTS

Z01 AR 41095-03 ARB

Z01 AR 41096-03 ARB

Z01 AR 41099-03 ARB

Z01 AR 41100-02 ARB

Z01 AR 41101-01 ARB

Z01 AR 41102-02 ARB

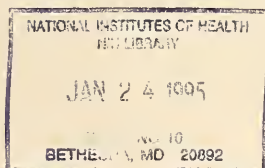
Z01 AR 41103-02 ARB

Z01 AR 41104-02 ARB

Z01 AR 41105-01 ARB

Z01 AR 41084-05 LSB

Z01 AR 41085-05 LSB





## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Mechanism of Muscular Contraction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Podolsky Chief LPB, NIAMS

Shigeru Chaen Visiting Scientist LPB, NIAMS

## COOPERATING UNITS (if any)

Dr. Robert Horowitz, Muscle Biophysics Section, LPB; Dr. Alasdair Steven, LSBR, NIAMS; Dr. Ellis Kempner, Macromolecular Biophysics Section, LPB; Dr. Neal Epstein, NHLBI; Dr. Lameh Fananapazir, NHLBI.

## LAB/BRANCH

Laboratory of Physical Biology

## SECTION

Section on Muscle Biophysics

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

MECHANICAL AND BIOCHEMICAL PROPERTIES OF MUTANT MYOSIN IN MUSCLE FIBERS FROM PATIENTS WITH FAMILIAL HYPERTROPHIC CARDIOMYOPATHY (HCM).

The contractile force, the unloaded contraction velocity, and the ATPase activity were measured in calcium activated, skinned soleus muscle fibers in controlled fibers and in mutant. We obtained surgical biopsies of soleus muscle from control patients as well as from HCM patients carrying one of three single missense mutations in one copy of the  $\beta$ -MHC gene. We found that isometric tension was decreased by ~15% in slow fibers from patients carrying a mutation in amino acid #403, but was unchanged in slow fibers from patients carrying the other two mutations. In another set of experiments, we simultaneously measured isometric tension and ATPase activity of single skinned fibers. The ATPase rate was measured by the standard NADH fluorescence method, which uses pyruvate kinase and lactic dehydrogenase to stoichiometrically couple ATP hydrolysis to NADH oxidation. We found that while HCM tends to be associated with a decrease in isometric tension output, the associated myofibrillar ATPase activity is increased. Taken together, these results suggest that in mutation the mechanical and chemical activities in the myosin molecule are less tightly coupled than in the normal fiber.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27001-20 LPB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Muscle Contractility and Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark Schoenberg, M.D. Medical Officer LPB, NIAMS

COOPERATING UNITS (if any)

Dr. Vincent Barnett, Department of Physiology, University of Minnesota Medical School

LAB/BRANCH

Laboratory of Physical Biology

SECTION

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treating muscle crossbridges in the presence of ATP with the alkylating agents para-phenylene dimaleimide (pPDM) or N-phenylmaleimide (NPM) destroys their ability to make force. Our evidence suggests that this treatment inhibits the purported force-producing conformational change by preventing the crossbridges from undergoing the weakly- to strongly-binding conformational change. Further evidence is presented which suggests that this alkylation effect is due to linking of phenylmaleimide groups to Cys-707 and Cys-697. Techniques are being developed to allow linking of phenylmaleimide groups to either Cys-707 or Cys-697 independently, so that these modified crossbridges can be tested for their ability to undergo the force-producing transition.

Also as part of these studies we developed a method for making the sarcolemmas of moderately large muscle fiber bundles sufficiently permeable and demonstrated, using a highly-sensitive ATPase assay, the efficacy of this technique for fiber bundles as large as 0.8 mm.





## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical Studies of Metabolic Activity and Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ellis S. Kempner, Ph.D.      Physicist      LPB, NIAMS

## COOPERATING UNITS (if any)

Drs. E. Dennis (UCSD); C. Hirschberg (Univ. Mass); G. Kaczorowski (Merck); J. Langer (UMDNJ).

## LAB/BRANCH

Laboratory of Physical Biology

## SECTION

Macromolecular Biophysics Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

## TOTAL STAFF YEARS:

2.0

## PROFESSIONAL:

1.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Target analysis of the inactivation of biological activity by ionizing radiation was applied to several major problems including cocaine receptors in brain, various enzymes, and energy transfer between synthetic polymers.

Objectives: 1) An understanding of the nature of active structures in vivo which are involved in biochemical processes, principally by means of the technique of inactivation by ionizing radiation. 2) Detailed knowledge of the molecular damage caused by ionizing radiation and of the mechanisms of the transfer of radiation energy throughout these structures.

Methods: 1) General biochemical techniques including enzyme reactions, fluorescence, and gel electrophoresis. 2) Ionizing radiation, usually high energy electrons from a linear accelerator, to expose samples under carefully controlled conditions.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Dynamic Properties of Cell Membranes and Related Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Norman Gershfeld, Ph.D. Research Chemist LPB, NIAMS

Others: Kazuhiro Fukada, Ph.D. Visiting Fellow LPB, NIAMS

## COOPERATING UNITS (if any)

Dr. L. Ginsberg, Dept. of Neurological Sciences, Royal Free Hospital, School of Medicine, University of London; Dr. C.P. Mudd, ACES, BEIP, NCRR; Dr. L.C. Yu, NIAMS

## LAB/BRANCH

Laboratory of Physical Biology

## SECTION

Macromolecular Biophysics Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

## TOTAL STAFF YEARS:

3.0

## PROFESSIONAL:

2.0

## OTHER:

1.0

## CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular mechanisms by which membranes assemble, and the conditions which determine their stability in healthy cells and in disease are the main focus of this study. We have previously reported that the lipid bilayer of membranes assembles and is optimally stable at a critical point, the physiological temperature of the cell. The stability of the membrane is extremely sensitive to changes in ambient temperature and in lipid composition resulting from defective lipid metabolism. Membrane degeneration follows as a natural consequence when these critical conditions are violated. Because of the possibility that measurement of  $T^*$ , the critical bilayer assembly temperature, may be useful for diagnosing lipid defects in membranes, this past year we have focussed on developing a rapid method for measuring the critical bilayer temperature. We have succeeded in developing an instrument for the rapid determination of  $T^*$  which utilizes microgram quantities of membrane lipid.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural and Mechanical Properties of Muscle Fibers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Leepo C. Yu, Ph.D. Research Physicist LPB, NIAMS

Others: Sengen Xu, Ph.D. Visiting Associate LPB, NIAMS  
Sergey Malinchik, Ph.D. Visiting Associate LPB, NIAMS  
Suzanne Frisbie, Ph.D. IRTA LPB, NIAMS  
Daniel Gilroy Mathematician LPB, NIAMS

## COOPERATING UNITS (if any)

Medical School of Hannover, FRG (Drs. B. Brenner and T. Kraft); East Carolina University Medical School, North Carolina (Dr. J. Chalovich); Duke University (M. Reedy); NIAMS, (Dr. Gershfeld)

## LAB/BRANCH

Laboratory of Physical Biology

## SECTION

Muscle Biophysics Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

## TOTAL STAFF YEARS:

5.0

## PROFESSIONAL:

4.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We have continued our efforts to understand the structural aspect of muscle contraction. During FY 94 we have obtained two dimensional diffraction patterns from relaxed muscle at low and high ionic strengths and at temperatures ranging from 4 degrees celcius to 30 degrees celcius. The actomyosin cross-bridges formed in the relaxed muscle have been shown to be in the precursor state to force generation. Under a wide range of conditions, we found that the integrated intensities of the myosin based layerlines are not affected by the extent of the cross-bridge attachment in the precursor, weakly bound state. This is consistent with the idea that the cross-bridge binding to actin is through the flexible part of the actin and/or of the myosin as suggested by Rayment, et al. (Science, 1993).

Osmotic compression applied to muscle fibers yields information on the lateral elastic property of the attached cross-bridges. Such information can discriminate structural differences among the cross-bridge states. We have continued using this technique showing that the cross-bridges attached to actin undergo a structural change upon activation. This work was carried out by using synchrotron sources in Germany and in the United Kingdom.

Modelling of the previously observed equatorial x-ray diffraction intensities suggest that the transition into force generating state is accompanied by a radial shift of mass by 5-10 Angstroms; the results are also consistent with the idea that the cross-bridges have less angular freedom during force production than in the weakly bound state.

Nucleotide (GTP) titration in the presence and absence of calcium requires a wide range of concentrations to reach full saturation. This can be accounted for if the nucleotide binding to myosin is a function of strain sustained by the attached cross-bridges.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Myofibril Assembly and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Robert Horowitz, Ph.D. Research Biologist LPB, NIAMS

Others:	Gang Luo, Ph.D.	Visiting Fellow	LPB, NIAMS
	Jian Qiao Zhang, Ph.D.	Visiting Fellow	LPB, NIAMS
	Tuyet-Phuong Nguyen	Summer IRTA Fellow	LPB, NIAMS

## COOPERATING UNITS (if any)

Dr. Richard Podolsky, NIAMS; Dr. Neil Epstein, NHLBI; Dr. Lameh Fananapazir, NHLBI;  
Dr. James R. Sellers, NHLBI

## LAB/BRANCH

Laboratory of Physical Biology

## SECTION

Muscle Biophysics Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

3.2

## PROFESSIONAL:

3.2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As a first step to studying the function of extremely large myofibrillar proteins using molecular genetic approaches, genes encoding these proteins must be isolated and cloned. In an attempt to isolate a clone carrying large portions of the coding sequence for mouse nebulin, a cDNA library was constructed using conditions designed to optimize the chances of cloning very large cDNAs. A putative nebulin cDNA clone has been isolated from this library. The clone carries an insert approximately 18 kilobases in length. Control experiments indicate that the entire insert is uncontaminated by the vector sequence, and hence must be genuine cDNA. We are currently mapping the insert for restriction endonuclease sites, and subcloning and sequencing selected segments.

Several distinct mutations in the beta-myosin heavy chain (MHC) gene have been linked to hypertrophic cardiomyopathy (HCM). Because the cardiac beta-MHC is also expressed in slow-twitch fibers of skeletal muscle, we have been able to study the mutant beta-myosin content and mechanical properties associated with these myosin mutations in single skinned skeletal muscle fibers obtained from HCM patients. We found that in patients carrying a mutation in amino acid #403 in one copy of the beta-MHC gene, the mutant beta-MHC comprises 47.3 +/- 9.1% of the total beta-MHC present in single slow twitch fibers. In one kindred in which muscle fibers from several individuals were studied, active tension decreased by a factor of two as mutant beta-MHC content increased from 32% to 65% of the total. These results suggest that the inherent ability of the mutant beta-myosins to generate isometric tension may be significantly decreased in some cases. Further studies indicate that the myofibrillar ATPase measured during isometric contraction is increased in HCM. Taken together, these results point to an increase in the energetic cost of producing tension in HCM.





## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Biology of Macromolecular Structure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alasdair Steven Chief LSBR, NIAMS

## Others:

Frank Booy	Visiting Scientist	Mario Cerritelli	IRTA Fellow
James Conway	Visiting Associate	Benes Trus	Guest Researcher
Manoj Misra	Visiting Associate	Michal Jarnik	Visiting Fellow
Naiqian Cheng	Visiting Associate	Martin Kessel	Special Volunteer
Eva Kocsis	Visiting Associate	Jose Gaston	Special Volunteer

## COOPERATING UNITS (if any)

Div. Computer Res. &amp; Tech., NIH; Dept. of Microbiology, Univ. Lab. (Drs. J. Wall, M. Simon, J. Hainfeld); others as noted.

## LAB/BRANCH

Laboratory of Structural Biology Research

## SECTION

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

10.25

## PROFESSIONAL:

10.25

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This Laboratory seeks to elucidate the mechanisms that govern the assembly of macromolecular complexes and the folding of macromolecules, with particular emphasis on their functional rationale. Over the past year, a main focus has been on conformational changes of viral capsid proteins. These conformational changes take place in space and time. L-A virus of yeast has been found to be the first virus to have a 120-subunit capsid made up of 60 symmetric dimers. In its precursor state, bacteriophage HK97 exhibits the remarkable feature of hexons that are sheared by a 25-A disclination into double trimers; and this feature disappears completely in the large-scale conformational transition that accompanies maturational expansion of the capsid. Herpes simplex virus type 1 deploys the same protein at both its hexon and penton sites, but the penton conformation, unlike the hexon conformation, does not permit binding of the 12kDa viral protein, VP26. New results have also been obtained on the long tail-fibers of bacteriophage T4, the receptor-recognizing organelle of this virus. By analyzing scanning transmission electron micrographs, we have found that, contrary to long-held opinion, these molecules are trimers (not dimers) and are organized in a novel, modular, filamentous structure.



## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## High-Resolution Structure and Function of Biological Macromolecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Craig Hyde	Expert	LSBR, NIAMS
Others:	Steven L. Edwards	Senior Staff Fellow	LSBR, NIAMS
	Joseph P. Mack	Senior Staff Fellow	LSBR, NIAMS
	Timothy C. Mueser	IRTA Fellow	LSBR, NIAMS
	Vaidehi Sridhar	IRTA Fellow	LSBR, NIAMS

## COOPERATING UNITS (if any)

LBP/NIDDK (N. Nossal), DCBDC/NCI (M. Maurizi), ARB/NIAMS (P. Plotz), OD/PEL (P. Wingfield, S. Stahl), St. Louis University (D. Grandgenett), NIAID/LIR (U. Siebenlist)

## LAB/BRANCH

Laboratory of Structural Biology Research

## SECTION

Hyde Working Group

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

5.0

## PROFESSIONAL:

5.0

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This Working Group studies the high-resolution structure and function of proteins and other macromolecules using x-ray diffraction methods. Although the groups' research interests are broadly based in all aspects of protein and nucleic acid structure, a common theme among the research subjects are macromolecular assemblies and complex interacting systems. Other aspects of the work are directed in basic science research on the structure of retroviral proteins and host cellular factors involved in HIV expression. Having been established less than three years ago, the group is now staffed at full strength and nearly fully equipped with modern instrumentation, computing, and equipment. Much effort has been expended to obtain large quantities of pure proteins and to obtain crystals suitable for high resolution x-ray diffraction studies. Work in the retroviral areas has focused on integrase, an enzyme that is essential in the retroviral life-cycle and an important target enzyme for therapeutic intervention. Because integrase from HIV has earned a reputation among protein chemists for being insoluble and intractable, our efforts have focused on the closely related enzyme from Rous sarcoma virus. An efficient means of producing 100 mg quantities of pure integrase using a bacterial expression system is now producing highly soluble and active enzyme which compares favorably in specific activity to material purified from live virus (D. Grandgenett). We have also produced large quantities of two forms of the human transcription factor NFkB. NFkB and its relatives are cellular factors of prime importance in understanding HIV expression. Crystallization trials underway for both integrase and one form of NFkB are very promising. Several crystal forms of human histidyl tRNA synthetase, an auto-antigen involved in the inflammatory disease polymyositis, have been obtained but are not yet suitable for diffraction analysis. Four other protein structures determinations underway in the lab include: the CLP-P complex, a large protease complex regulated by an ATP-binding subunit complex; two protein of the T4 phage DNA replication complex (GP 59 and RNase H), and a bacterial phosphoribosyl transferase. All four structures are novel and will likely require the use of multiple heavy-atom isomorphous replacement methods for phasing.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Cell Surface Receptor for IgE

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Henry Metzger, MD Chief, Chemical Immunology Section ARB/NIAMS

Su-Yau Mao, PhD Visiting Associate

Ute Kent, PhD IRTA Fellow

Victor Pribluda, PhD Visiting Scientist

Clara Pribluda, MD Visiting Fellow

Becky Vonakis, PhD IRTA

T Yamashita, PhD Visiting Fellow

## COOPERATING UNITS (if any)

David Holowka, Barbara Baird, E.-Y. Chang (Cornell University)

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Chemical Immunology Section

## INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N258, Bethesda, MD 20892

## TOTAL STAFF YEARS:

6.6

## PROFESSIONAL:

6.6

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The high affinity receptor for IgE on mast cells & basophils (FcEpsilonRI) plays a central role in immediate hypersensitivity reactions. Reaction of receptor-bound IgE with polyvalent antigen clusters the receptors & this stimulates cellular secretion of both preformed & newly synthesized mediators of inflammation. The molecular mechanisms by which aggregation of the receptors generate these cellular responses are the focus of our studies. The close relationship of FcEpsilonRI to other receptors central to the functioning of the immune system (e.g. the clonotypic receptors on T & B lymphocytes), make it likely that the significance of such studies extends beyond the IgE/mast cell system. During the past year, our principal progress was in three areas: 1) Identifying additional components that become chemically crosslinked to resting & aggregated receptors. Of the large number of candidate proteins that were examined two showed significant association with FcEpsilonRI: p53/56lyn kinase & protein kinase C delta. Collaborative studies of the rotational diffusion of FcEpsilonRI were completed & are consistent with interactions of the FcEpsilonRI with substantial amounts of other cellular components. 2) Exploring further various aspects of the association between tyrosine kinase activity & the receptor. Using either gentle isolation procedures or chemical crosslinking, we obtained further evidence that transphosphorylation is the fundamental mechanism by which aggregation of FcEpsilonRI both initiates & amplifies the cascade of tyrosine phosphorylations. Evidence for early recruitment of additional kinase was also obtained. 3) Completing studies on the dynamics of signal transduction in vivo. Contrary to the proposals of others, our studies some of which were published this year, indicate that aggregated receptors maintain their ability to signal for prolonged periods in a dynamic process. We collected further data that will allow us to characterize this system quantitatively.





## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bacterial Cell wall-induced arthritis and hepatic granuloma formation in the rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.L. Wilder	Chief	
Y. Du	Visiting Fellow	
E. Remmers	Sr. Staff Fellow	All IJDS, ARB/NIAMS
H.B. Zha	Visiting Associate	
K. Kalogeras	Sr. Staff Fellow	
J. Wells	Staff Fellow	
Lyn Ge	IRTA Fellow	
S. Kotake	Visiting Fellow	

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD  
Holland Labs, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

2

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lewis rats are highly susceptible to bacterial cell wall arthritis, but Fischer rats are relatively resistant. We have previously provided data suggesting that this difference was related to blunted hypothalamic-pituitary-adrenal axis (HPA) responsiveness in the Lewis rat. We have also demonstrated that HPA axis responses to LPS differ markedly in the 2 rat strains. Our studies over the past year have focused on the hypothesis that the differences in the hormonal responses of these rat strains should be reflected in differences in cytokine production. We have developed PCR-based assays to quantitate the production of numerous cytokines including gamma-interferon, interleukin-4, -2, -6 and -10. Studies are in progress to measure each of these cytokines and their relationship to hormone secretion in response to LPS.





## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of synovial tissues from patients with RA and related conditions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.L. Wilder

Chief

E. Remmers

Sr. Staff Fellow

All IJDS, ARB/NIAMS

K. Kanik

Med. Staff Fellow

## COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD  
Holland Labs, American Red Cross

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Inflammatory Joint Diseases Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

0.5

## PROFESSIONAL:

0.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Animal model studies from our lab have indicated that neuroendocrine mechanisms play a major role in regulating inflammatory processes that are operative in chronic proliferative forms of arthritis. We are exploring these concepts in patients with rheumatoid arthritis (RA). We have demonstrated high levels of expression of corticotropin releasing hormone (CRH) in synovial fluids and tissues from patients with RA.

We have also demonstrated that cyclooxygenase -1 and -2 are both expressed in rheumatoid synovia, but only Cox-2 is inducible. Interleukin-1 and PMA are potent stimulators of Cox-2, but not Cox-1. Corticosteroids suppress Cox-2, but not Cox-1 expression. These data support the view that Cox-2, like CRH, may mediate signals between the neuroendocrine and inflammatory systems. Dysregulated expression of the polypeptides appears to be associated with inflammatory joint disease.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on etiology and pathogenesis of idiopathic inflammatory myopathy in humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB/NIAMS  
Frederick Miller, M.D., Ph.D., Special Volunteer, ARB/NIAMS  
Nina Raben, M.D., Ph.D., Visiting Scientist, ARB/NIAMS  
Ralph Nichols, Ph.D., Staff Fellow, ARB/NIAMS  
Jan Dohlman, M.D., Senior Staff Fellow, ARB/NIAMS  
Elizabeth Adams, M.D., Clinical Associate, ARB/NIAMS  
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB/NIAMS  
Lisa Rider, M.D., CBER/FDA

COOPERATING UNITS (if any)

Dr. Terry O'Hanlon, CBER/FDA  
Dr. Craig Hyde, LSB/NIAMS

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Building 10 Room 9N244, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.2

PROFESSIONAL:

5.2

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Idiopathic inflammatory myopathy (polymyositis, dermatomyositis, and related disorders) is a family of inflammatory diseases in which disease-specific autoantibodies occur and for which there is considerable indirect evidence pointing to a viral etiology. We have over the past several years, seen and studied and collected serum, blood, and muscle specimens from well over 450 patients suspected of having myositis and we have collected epidemiologic information on many patients.

We have located the principal epitope for autoantibodies in the amino-terminus of HRS. This region has been shown to be a coiled-coil by circular dichroism study of fragments of the region- a result predicted by a computer analysis. Furthermore, this analysis has uncovered structural homology to the aminoterminus of several other aminoacyl-tRNA synthetases.

In collaboration with Dr. Terry O'Hanlon, we have studied the primary sequence of  $\alpha\beta$  T-cell receptor of polymyositis, dermatomyositis and inclusion body myositis.

In collaboration with several colleagues in other cities, we have described the spectrum of autoimmunity in juvenile myositis.

Dr. Sherman has made major progress in developing lymphocytes which recognize muscle cell-specific autoantigens. Dr. Adams has developed assays to measure cytokine mRNA levels in the muscle biopsies and peripheral blood cells of myositis patients.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 41076-07 ARB

## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Therapeutic trials in idiopathic inflammatory myopathies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB/NIAMS  
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB/NIAMS  
Frederick Miller, M.D., Ph.D., Special Volunteer, ARB/NIAMS  
Elizabeth Adams, M.D., Clinical Associate, ARB/NIAMS  
Lisa Ginn, M.D., IRTA, ARB/NIAMS

## COOPERATING UNITS (if any)

Jeanne Hicks, M.D., CC Rehabilitation  
Melissa Bartlett, M.S., CC Radiology  
Steve Bacharach, Ph.D., CC Radiology

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Connective Tissue Diseases Section

## INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

## TOTAL STAFF YEARS:

1.1

## PROFESSIONAL:

1.1

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In an attempt to find a better way to treat steroid-resistant myositis (other than inclusion body myositis), we have carried out a randomized crossover trial of intravenous methotrexate with leukovorin rescue and a combination of methotrexate and azathioprine. Accrual of patients is complete and analysis will be within the next year.

We have begun a trial of the purine analogue, fludarabine, in the therapy of myositis. A quantitative method of assessing muscle inflammation in myositis has been developed and will be used in current and future therapeutic trials.



PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Familial Mediterranean Fever

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Daniel L. Kastner, M.D., Ph.D., Acting Chief, Genetics Section, ARB/NIAMS  
Ivona Aksentijevich, M.D., IRTA Fellow, ARB/NIAMS  
Xiang Chen, Ph.D., IRTA Fellow, ARB/NIAMS  
Raman Sood, Ph.D., IRTA Fellow, ARB/NIAMS  
Ernesto Levy, M.D., Visiting Fellow, ARB/NIAMS  
Geryl Wood, Biologist, ARB/NIAMS  
Christopher F. Mojcik, M.D., Ph.D., Staff Fellow, LI/NIAID  
Pu Liu, M.D., Ph.D., Senior Staff Fellow, LGT/NCHGR

COOPERATING UNITS (if any)

Lab of Immunol, NIAID; Los Alamos Natl Lab; Lab of Gene Transfer, NCHGR;  
Adelaide Children's Hospital, Australia; Dept. Pediatrics, Cedars-Sinai Hospital;  
Heller Institute, Israel; Dept. Anatomy, Univ of Michigan; Vollum Inst, Oregon;

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892-1816

TOTAL STAFF YEARS:

7.0

PROFESSIONAL:

4.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Familial Mediterranean fever (FMF) is a rheumatic disease characterized by intermittent bouts of fever with abdominal pain, pleurisy, and/or arthritis; some patients also develop systemic amyloidosis, leading eventually to renal failure. FMF is inherited as a single autosomal recessive gene (designated MEF), but the biochemical lesion is unknown. The purpose of this project has been to identify the FMF gene by positional cloning. By the beginning of the current year, we had mapped the gene causing FMF to a 7 cM interval on the short arm of chromosome 16. During 1993-94, our efforts were divided among genetic mapping, cloning the relevant region of chromosome 16, analysis of candidate genes, and functional studies.

In collaboration with investigators at Adelaide Children's Hospital and the University of Michigan, we have identified 5 new microsatellite markers that lie in the previously defined MEF candidate region. By genotyping a panel of 47 non-Ashkenazi Jewish FMF families, we found that all 5 markers are centromeric to MEF. There was only one detectable recombinant between the closest of these markers and MEF, versus 11 recombinants for D16S63, the closest centromeric marker known one year ago. Moreover there is only a single recombinant for D16S246, the telomeric flanking marker. We have found highly significant linkage disequilibrium at both ends of the MEF interval, as it is presently defined.

In order to clone the chromosomal region identified by these flanking markers, we developed sequence-tagged sites (STSs) for the flanking cosmids as well as other cosmids that have been physically mapped to the appropriate area of chromosome 16. In collaboration with workers at NCHGR, Los Alamos, and the University of Michigan, we screened libraries of human genomic DNA cloned in yeast artificial chromosomes (YACs). We have identified 5 YAC clones that contain the region of interest. The smallest has an insert size of 680 kb.

To date three genes have been identified from MEF candidate region. The genes encoding heme oxygenase-2 (HMOX2) and CREB-binding protein (CBP) have been excluded from further consideration as the MEF region was narrowed over the last year. A third apparently novel expressed sequence is currently under investigation.

To evaluate positionally-defined candidate genes, a better understanding of the functional abnormalities in FMF is useful. In collaboration with members of the LI/NIAID, we have found that peripheral blood leukocytes from FMF patients show increased levels of adhesion to fibronectin and type I collagen, relative to normals.







## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Neuroendocrine Factors in the Autoimmune Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.L. Wilder Chief, IJDS  
K. Kalogeras Sr. Staff Fellow  
K. Kanik Med. Staff Fellow

## COOPERATING UNITS (if any)

G. Chrousos, Developmental Endocrinology Br, NICHD

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Inflammatory Joint Diseases Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

.5

## PROFESSIONAL:

.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies from animal models have suggested the possibility that hypothalamic-pituitary-adrenal axis and other neuroendocrine dysfunctions may play a role in rheumatoid arthritis and other autoimmune conditions. These animal studies have also suggested that HPA axis dysfunction may predispose an individual to many different types of autoimmune disease. For these reasons, studies are in progress to evaluate neuroendocrine function in patients with rheumatoid arthritis and related conditions. Our initial studies support the hypothesis that HPA axis responsiveness is blunted in patients with new-onset rheumatoid arthritis. Over the past year, we have continued to collect data, but analysis is not complete. We have also completed an evaluation of HPA axis and sympatho-adrenal function in fibromyalgia patients. These patients exhibit blunted HPA axis responses to corticotropin-releasing hormone stimulation and markedly depressed plasma levels of neuropeptide Y. These data suggest that fibromyalgia patients have blunted function of the two major components of the stress response system. The cause of these abnormalities is not yet clear.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mapping of Genes and Genetic Polymorphisms in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.L. Wilder	Chief, IJDS	
Y. Du	Visiting Fellow	
E. Remmers	Sr. Staff Fellow	All IJDS, ARB/NIAMS
H.B. Zha	Visiting Associate	
Lyn Ge	IRTA Fellow	
S. Kotake	Visiting Fellow	

## COOPERATING UNITS (if any)

M. Griffiths, Univ. of Utah

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Inflammatory Joint Diseases Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

3

## PROFESSIONAL:

3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rats are an important experimental model for many human diseases, many of which have a genetic component. As followup of our previous and ongoing work demonstrating impressive differences in the phenotypic responses of LEW and F344 rats to proinflammatory and noninflammatory stimuli, we are developing a genetic linkage map for rats, specifically applicable to these rat strains. We have now identified approximately 400 polymorphisms and have mapped about 360 of these to 19 of 20 rat chromosomes using an F2 cross of F344 x LEW parents. We have also mapped 155 markers using an F2 cross of LEW x BN parents. We have demonstrated impressive conservation of synteny between rat, mouse and human chromosomes. We have also extended our efforts to identify polymorphisms to 13 additional inbred strains of rats. In summary, we have continued to make progress in our efforts to develop a genetic linkage map for rats, which is a prerequisite for chromosomally localizing genes that control important phenotypic traits in rats. Moreover, these markers are highly useful for genetic monitoring of inbred rat strains.

Part of our efforts have been focused on mapping the athymic nude gene in the rat. We have now mapped this gene within one centimorgan of the nitric oxide synthase gene on chromosome 10. We have also made an effort to map the rat osteopetrosis gene. Preliminary data suggest that we have identified its chromosomal location, but more data are needed for confirmation. Efforts to map autoimmune disease susceptibility and resistance genes in crosses of Lewis and F344 rats are in progress.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AR 41095-03 ARB

## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Translational and rotational power terms in 6 degree-of-freedom joint modeling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.P. Holden, IRTA Fellow, Biomechanics Laboratory, ARB/NIAMS

S.J. Stanhope, Director, Biomechanics Lab (BML), RMD/CC

T.M. Kepple, Programmer Specialist, BML, RMD/CC

K.L. Siegel, Senior Staff Therapist, BML, RMD/CC

## COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

## INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

## TOTAL STAFF YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An engineering methodology called "inverse dynamics" provides the resultant forces and moments at major anatomical joints during walking. These "joint reactions" perform mechanical work, and consequently have power associated with them. Joint powers have been used to evaluate mechanical energy flows for individual joints. If added for all major joints of the body, the sum represents a mechanical energy cost for movement, which is an important parameter for estimation of mechanical efficiency. Traditionally, joint models have allowed only rotations at joints, without accounting for translation such as sliding and compression/distraction. Our immediate objective is to implement full six degree-of-freedom (DOF) joint models for the lower extremity, allowing for three rotational and three translational movements (DOFs). Traditional models assumed that translational powers canceled each other across a joint; our new methods include joint powers associated with the three translational DOFs. We believe this detailed model will provide more robust calculations for joint powers, improving reliability and accuracy in total mechanical power estimated. Lower extremity data are collected over full stride cycles for multiple walking trials. Ensemble averages and coefficients of variability are determined for each DOF.

Results at the knee joint for an intra-subject analysis (n=5) showed that, as expected, the greatest peak magnitude in joint power occurred for the rotational DOF associated with flexion/extension. The next largest peak occurred for the vertical translational DOF, reaching 40% of the flexion/extension peak. Translational knee joint velocities were as high as 14% of the forward walking speed of 1.24 m/s. Mechanical work values during three power absorption bursts were significantly less for the 6 DOF model than for both 1 DOF (pin) and 3 DOF (ball-and-socket) rotational models. Inter-subject analyses (n=50) were conducted on joint power data, with similar results. These results at the knee joint were similar to those previously found at the ankle.



## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The genetic basis for metabolic myopathies.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB/NIAMS  
Nina Raben, M.D., Ph.D., Visiting Scientist, ARB/NIAMS  
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB/NIAMS  
Cornelius Boerkoel, III, M.D., Ph.D., IRTA, ARB/NIAMS  
Frederick Miller, M.D., Ph.D., Special Volunteer, ARB/NIAMS  
Elizabeth Adams, M.D., Clinical Associate, ARB/NIAMS  
Rachel Exelbert, Pre-doctoral IRTA, ARB/NIAMS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Connective Tissue Diseases Section

## INSTITUTE AND LOCATION

NIAMS, Building 10 Room 9N244, Bethesda, MD 20892

## TOTAL STAFF YEARS:

3.2

## PROFESSIONAL:

2.0

## OTHER:

1.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analysis of mutations and correlation with clinical illness has been greatly expanded by the analysis of a number of families with both phosphofructokinase (PFK) and acid maltase deficiencies. Plans for gene therapy of acid maltase deficiency have moved forward in several areas.

A number of metabolic/genetic myopathies have been diagnosed.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AR 41100-02 ARB

## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Skeletal tracking with skeletal fixation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.P. Holden

IRTA Fellow, Biomechanics Laboratory

ARB/NIAMS

M.C. Brown

IRTA Fellow, Biomechanics Laboratory

ARB/NIAMS

## COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)

S.J. Stanhope Dir., Biomechanics Lab (BML) RMD/CC

K.L. Siegel, Senior Staff Therapist &amp; T.M. Kepple, Programmer Specialist, BML

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

## INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0

## CHECK APPROPRIATE BOXES

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The use of gait analysis in clinical settings has become widespread. Evaluation of a patient's walking pattern requires the attachment of optical targets to the skin's surface. Data derived from monitoring the locations of these targets while the patient walks provide the basis for an objective study of the movement. The motion of the skin and underlying soft tissues relative to the bone is known to be a source of error in the skeletal motion measurement, but these errors are routinely ignored without any detailed knowledge of their magnitude. The purpose of this study is to evaluate the magnitude and pattern of error produced by tracking the skeletal system using skin mounted targets.

Two sets of optical targets are attached to each segment to be studied -- one set mounted to the surface and one mounted to the bone. A three-dimensional target tracking system is used to measure simultaneously the movement of the two target sets as the subject walks in the laboratory. The six degree-of-freedom motions of the segment are calculated using each target set, and the difference in motions represents the error introduced by the surface target attachment technique.

To date, the gait of three subjects has been tested using surface and bone mounted targets affixed to the shank (lower leg). The results indicate that kinematic errors occur primarily about and along the longitudinal axis, with peaks of 8 degrees rotation and 10 mm translation. Based on the error patterns during the gait cycle, it does not appear feasible to model these errors by taking into account only the passive motion of soft tissue.



PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Cystinuria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Daniel L. Kastner, M.D., Ph.D., Acting Chief, Genetics Section, ARB/NIAMS  
 Elon Pras, M.D., Visiting Fellow, Genetics Section, ARB/NIAMS  
 Nina Raben, M.D., Ph.D., Visiting Scientist, Connective Tissue Diseases/ARB/NIAMS  
 Eliahu Golomb, Ph.D., Visiting Fellow, Hypertension Endocrine Branch/NHLBI

COOPERATING UNITS (if any)

Connective Tissue Diseases Sec./ARB/NIAMS	Hadassah Medical Center, Jerusalem
Hypertension Endocrine Branch/NHLBI	Institut Pasteur, Paris, France
Heller Institute, Tel-Hashomer, Israel	Beilinson Medical Center, Israel

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892-1816

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cystinuria is an autosomal recessive disease in which there is excessive excretion of cystine in the urine, with precipitation of urinary cystine stones. Patients with this disorder may have renal colic, urinary tract obstruction, secondary urinary tract infection, and, if untreated, renal insufficiency. The chromosomal location of the cystinuria gene and the molecular basis of cystinuria were unknown at the initiation of this project. The purpose of this project was to identify the chromosomal location of the cystinuria gene.

Our strategy was to obtain DNA specimens from cystinuria families with more than one affected member, and then to test for genetic linkage between known markers and the inheritance of the disease. We obtained specimens from 17 families (113 individuals, 44 affected) from Israel and the U.S. In most cases Epstein-Barr virus-transformed B cell lines were established; genomic DNA was isolated from cell lines and/or freshly isolated lymphocytes.

Genetic markers were chosen from microsatellites developed by Dr. Jean Weissenbach (Institut Pasteur and Genethon). Although we had initially observed one family suggesting linkage to chromosome 16, study of a larger panel of families, with more markers, excluded linkage anywhere on this chromosome. A subsequent genome-wide search of over 50 microsatellites also failed to establish linkage.

About one year ago, a human kidney cDNA whose protein product is involved in cystine, dibasic, and neutral amino acid transport was mapped to chromosome 2. After testing several microsatellites, we found linkage between cystinuria and 3 chromosome 2p markers (D2S119, D2S391, and D2S288), with a maximal pairwise lod score over 8. Analysis of recombinants indicated the most likely order to be cen-D2S391-D2S119-cystinuria-D2S177-tel. We also observed high rates of homozygosity for markers in this chromosomal region among 11 affected offspring of inbred marriages. There was no evidence for locus heterogeneity among our 17 families.

We then looked for mutations in SLC3A1 (the aforementioned amino acid transporter) in our cystinuria families. In one American family, we found a frameshift in the father and a large deletion in the mother. Simultaneous with the publication of our chromosome 2 linkage data, another group reported 6 missense SLC3A1 mutations in cystinuria patients. Screening of our 16 additional families did not reveal any of these missense mutations or the frameshift mutation we had found. This suggests that there are additional SLC3A1 mutations yet to be found.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AR 41103-02 ARB

## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Genetics of Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Daniel L. Kastner, M.D., Ph.D., Acting Chief, Genetics Section, ARB/NIAMS  
 Michael McDermott, M.D., Visiting Scientist, Genetics Section, ARB/NIAMS

## COOPERATING UNITS (if any)

Department of Rheumatology  
 University College  
 Cork, Ireland

Department of Human Genetics  
 University of Utah

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

Genetics Section

## INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892-1816

## TOTAL STAFF YEARS:

.75

## PROFESSIONAL:

.75

## OTHER:

0

## CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rheumatoid arthritis (RA) is a chronic peripheral arthritis of unknown etiology affecting 1-2% of the world's population. Studies of identical twins and multiplex families strongly suggest a genetic component of RA susceptibility, but these same studies point to multiple genes and reduced penetrance. The purpose of this project was to test the role of certain candidate genes in RA susceptibility.

Population studies have shown that one susceptibility locus is probably associated with the HLA-DR4 and DR1 alleles of the major histocompatibility complex (MHC). Since HLA-DR molecules are known to present antigen to the T-cell receptor (TCR), we speculated that TCR genes might also contribute to RA susceptibility. In collagen-induced arthritis, a murine model of RA, disease susceptibility is jointly determined by MHC and TCR genes. We therefore decided to study the role of TCR genes in determining RA susceptibility in man.

DNA specimens were obtained from multiplex RA families seen in rheumatology clinics in Cork, Salt Lake City, and Toronto. A total of 28 families (215 members, 79 affected) were studied. Families were genotyped for markers associated with the TCR beta and gamma chains, both by Southern blotting and by the polymerase chain reaction. Data were analyzed by a linkage analysis method that allowed for more than one susceptibility locus, and by affected sib-pair analysis.

During this study we obtained suggestive, but not conclusive, evidence that genes linked to the TCR beta chain locus may encode RA susceptibility. For a V-beta 12.2 SSCP marker, there was 61% sharing of alleles in affected sib-pairs, versus an expected value of 50% ( $p = 0.005$ ; a  $p$  value of 0.001 is generally taken to establish linkage for this test). There was 63% sharing of V-beta 6.7 microsatellite alleles ( $p = 0.06$ ), but only 53% sharing for a less informative C-beta RFLP ( $p = 0.19$ ). We calculated that, for the two more informative markers, a homogeneous group of more than 100 similar families would be needed to reach statistical significance.

We also studied the potential contribution of genes linked to the TCR gamma locus by genotyping our families for the highly informative D7S485 microsatellite marker. By affected sib-pair analysis, there was no significant sharing of TCRG alleles ( $p = 0.28$ ). Moreover, using conventional linkage analysis, there were significantly negative lod scores (i.e. less than -2.0) for both dominant and recessive models.





## PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies of the Rheumatic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John H. Klippel, M.D., Clinical Director, NIAMS

Mark Gourley, M.D., Senior Staff Fellow, ARB/NIAMS

Dorothy Scott, M.D., Special Volunteer, ARB/NIAMS

H. Ralph Schumacher, M.D., IPA, U. Pennsylvania

## COOPERATING UNITS (if any)

J. Pando, Medical Staff Fellow, ARB/NIAMS

C. Yarboro, Research Nurse, ARB/NIAMS

F. Pucino, Pharmacy, CC

T. Fleisher, Clinical Immunology, CC

D. Faustman, Massachusetts General

Hospital, Boston

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

## TOTAL STAFF YEARS:

2

## PROFESSIONAL:

2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have conducted a series of clinical investigations involving patients with systemic lupus erythematosus - a chronic, relapsing and remitting disorder characterized by immune-mediated inflammation. A large population of patients with systemic lupus has been entered into a natural history study in which questions related to disease pathogenesis, epidemiology, and co-morbidity are addressed. Therapeutic studies have mainly focused on long-term, randomized trials of patients with severe, proliferative lupus glomerulonephritis and the development of phase I/II studies of newer approaches to disease management.





## PERIOD COVERED

January 3, 1994 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of Walking Speed on the Mechanics of Gait

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.P. Holden, IRTA Fellow, Biomechanics Laboratory, ARB/NIAMS

## COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH  
S.J. Stanhope, Director, Biomechanics Laboratory, RMD/CC

## LAB/BRANCH

Arthritis and Rheumatism Branch

## SECTION

## INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

## TOTAL STAFF YEARS:

0.5

## PROFESSIONAL:

0.3

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gait analysis is being applied increasingly in clinical settings to provide input to surgical decisions and rehabilitation treatment planning. Gait analysis utilizes measured movement patterns and ground reaction forces in a biomechanical model of the body to investigate the neuromuscular strategies used to produce the movement. One set of useful variables are net joint moments, which represent the net effect of forces (primarily muscle forces) acting to either produce or resist joint rotations. Net muscle moment patterns are used to evaluate patient neuromuscular function, often through comparison with average patterns produced by healthy individuals. Patients being evaluated for gait abnormalities, however, routinely walk at slower than normal speeds, which is known to affect many gait parameters. Thus, it is important to distinguish variations due to differences in walking speed from variations due to true gait abnormalities or adaptations. For example, the effect of different walking speeds on the net flexion-extension moment pattern at the knee is not clear, yet studies of patients with ACL deficiency have reported a "quadriceps avoidance" gait adaptation without addressing the issue of walking speed. The purpose of this project is to investigate the effect of dramatically different walking speeds on the mechanics of walking in healthy adults. One specific objective is to determine if a "quadriceps avoidance" pattern is demonstrated by healthy subjects at slower walking speeds. The results have implications for the interpretation of patient gait data.

Movement and force data are collected as subjects walk at five different speeds. Subjects are required to walk at 25%, 50%, 75%, 100% and 125% of a normalized walking speed of 0.785 statures/s ( $\pm 2.5\%$ ). The three-dimensional movements of the lower limb are measured using a video-based (50 Hz) system to track retroreflective targets attached to each segment, and a force platform measures ground reaction forces at 200 Hz. Net knee joint moments are calculated during the stance phase, and ensemble averages and estimates of variability are determined for each speed.



## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Features of Keratin and Related Intermediate Filaments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert, Ph.D.	Chief	LSB, NIAMS
Others:	John G. Compton, Ph.D.	Senior Staff Fellow	LSB, NIAMS
	James W. Mack, Ph.D.	Special Volunteer	LSB, NIAMS
	Lyuben Marekov, Ph.D.	Visiting Scientist	LSB, NIAMS
	Jun-Mo Yang, Ph.D.	Visiting Fellow	LSB, NIAMS

## COOPERATING UNITS (if any)

RD Goldman, Prof & Chair, Dept of Cell, Mol & Struct Biol, Northwestern Univ Med School, Chicago; ACT North, Prof, Dept of Biochem & Mol Biol, Univ of Leeds, Leeds, UK; DAD Parry, Prof, Dept of Physics & Biophy, Massey Univ, New Zealand

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Molecular Biology of Keratinization Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

3.5

## PROFESSIONAL:

3.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure, function and expression of keratin intermediate filaments of human skin, and the related intermediate filament proteins of other cell types and of cultured cells, are being investigated. These studies are designed to understand the structural features that determine how the rod domains of the constituent coiled-coil molecules pack together to form the filament core. We have used crosslinking studies to determine molecular lengths and nearest-neighbor alignments in epidermal keratin and vimentin filaments, and we have found that while common alignment principals are used, the exact alignments are slightly different. We have used modelling studies to predict a likely conformation of the L2 linker and 2B stutter regions of the coiled-coil rod of keratin molecules, two of the highly conserved disruptions in the structure of the coiled-coil rod that are likely to be important in determining molecular packing. We have also identified an important overlap of about 1 nm between the end of the rod domain of one molecule with the beginning of the rod domain of the next molecule in an axial row. We have used synthetic peptides to explore this overlap: residues 5-15 of the 1A segment at the beginning of the rod domain and residues 107-117 of the 2B segment at the end of the rod domain are most important. Interestingly, most mutations that cause skin diseases occur in these two overlapping sequences. Micro-injection of a synthetic 1A peptide into cultured cells causes massive reversible disassembly of the filament networks in both fibroblast (vimentin-containing) and epithelial (keratin-containing) cells, with concomitant changes in cell shape. In other studies, we have found that the V1 end domain sequence region of the keratin 1 chain is involved in crosslinking to the cell envelope, which therefore provides a mechanism by which the keratin intermediate filament network of terminally-differentiated epidermal cells is anchored to the cell periphery.



## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression, Structure and Function of Filaggrin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter M. Steinert, Ph.D.

Chief

LSB, NIAMS

Others: Jeanne Andreoli

IRTA Fellow

LSB, NIAMS

Medialka Markova

Visiting Scientist

LSB, NIAMS

Shyh-Ing Jang

IRTA Fellow

LSB, NIAMS

## COOPERATING UNITS (if any)

Dietmar Mischke, Staff Scientist, Free University of Berlin, Germany; Howard University, Washington, DC

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Molecular Biology of Keratinization Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

2.5

## PROFESSIONAL:

2.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Filaggrin, the processed form of profilaggrin, is a major product of terminally differentiated mammalian epidermal cells that is thought to be involved in the aggregation and specific alignment of keratin intermediate filaments during the final stages of differentiation. We have determined that filaggrin binds directly to the filaments by way of ionic interactions of the positive charges on a repeating  $\beta$ -turn motif of filaggrin with the frequent negative charges on the filaments. Having fully characterized the structure of the profilaggrin gene, we have now begun a systematic analysis of the regulatory sequences that control its expression, by use of reporter gene systems. The proximal promoter element region is located within the first 350 bp immediately above the cap site. In fact, the first 80 bp are sufficient to restrict transcription to terminally differentiating epidermal cells. This control is exerted through a complex set of interactions between an AP1 site and adjacent/overlapping etc, NF-kB and Sp1 DNA binding motifs. We have also coupled these sequence regions to a  $\beta$ -galactosidase reporter gene system and have constructed transgenic mice to explore the expression of the profilaggrin gene.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AR 41086-05 LSB

## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression, Structure and Function of Loricrin, a Major Cell Envelope Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert, Ph.D.	Chief	LSB, NIAMS
Others:	Eleonora Candi, Ph.D.	Special Volunteer	LSB, NIAMS
	Vincenzo DeLaurenzi, Ph.D.	Special Volunteer	LSB, NIAMS
	William Idler	Chemist	LSB, NIAMS
	Soo-Yool Kim, Ph.D.	Visiting Fellow	LSB, NIAMS
	James Mack, Ph.D.	Special Volunteer	LSB, NIAMS

## COOPERATING UNITS (if any)

A Finazzi-Agro, Genaro Melino, Giampiero Mei, Dept of Exp Med, Univ Tor Vergata, Rome, Italy; K Yoneda, Dept of Dermat, Faculty of Med, Kyoto Univ, Kyoto, Japan; C Backendorf, Staff Scientist, Lab of Mol Genetics, Univ of Leiden, Netherlands

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Molecular Biology of Keratinization Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

2.5

## PROFESSIONAL:

2.0

## OTHER:

0.5

## CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The cornified cell envelope constitutes a multi-component 15 nm thick layer of highly insoluble protein on the inside of the plasma membrane of terminally differentiated epithelial cells. This insolubility is largely due to crosslinking of the proteins by N<sup>ε</sup>-(γ-glutamyl)lysine isodipeptide crosslinks by the action of transglutaminases. Based on a variety of data, loricrin is the major component of the epidermal cell envelope. Following digestion of isolated envelopes with proteases, large numbers of crosslinked peptides were released, separated and more than 100 were then sequenced. The vast majority consisted of intra- and inter-chain loricrin-lovicrin crosslinks. Several others involved interchain loricrin-SPR1 or loricrin-SPR2, loricrin-keratin 1, loricrin-keratin 10 or loricrin-filaggrin crosslinks. The data support the notion that loricrin is the major component of the cell envelope and that it provides the attachment site by which the underlying cytoskeleton is anchored. Full-length human loricrin has been expressed in bacteria and purified to homogeneity. In vitro crosslinking with transglutaminases 1, 2 and 3 reveals that only transglutaminase 3 oligomerizes loricrin by way of isodipeptide crosslinks, all of which are identical to those seen from isolated native cell envelopes. We have also expressed the human SPR1, SPR2 and SPR3 proteins in bacteria for subsequent biochemical, crosslinking and structural analyses. In order to determine its precise role in the epidermis, we have used transgenic technology with the human loricrin gene to (a) perform dominant negative mutation analysis with a mutated form of the gene having coding sequences containing important crosslinking sites deleted; and (b) create loricrin-null mice.





## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Epidermal Transglutaminases**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert	Chief	LSB, NIAMS
Others:	Vincenzo DeLaurenzi	Special Volunteer	LSB, NIAMS
	William Idler	Chemist	LSB, NIAMS
	Shyh-Ing Jang	IRTA Fellow	LSB, NIAMS
	Soo-Yool Kim	Visiting Fellow	LSB, NIAMS
	Seung-Chul Lee	Visiting Fellow	LSB, NIAMS
	Jeung-Hoon Lee	Visiting Fellow	LSB, NIAMS
	Edit Tarcza	Visiting Fellow	LSB, NIAMS

## COOPERATING UNITS (if any)

Soo-Il Chung, Sen Inv, LCDO/NIDRO; Wesley McBride, Sen Inv, DCBDC/NCI; Sang-Chul Park, Prof, Dep of Bioch, Seoul Nat Univ Med Schl, Korea; In-Gyu Kim, Prof, Dep of Bioch, Inha Univ, Korea; Kozo Yoneda, Dep of Derm, Schl of Med, Kyoto Univ, Japan

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Molecular Biology of Keratinization Section

## INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

## TOTAL STAFF YEARS:

4.5

## PROFESSIONAL:

4.5

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transglutaminases form an isodipeptide crosslink between an acceptor amide group of a protein bound glutamine residue and a donor  $\epsilon$ -NH<sub>2</sub> group of a protein bound lysine residue, thereby forming a highly insoluble macromolecular complex. In the epidermis and other stratified squamous epithelial tissues, these enzymes are thought to be involved in the crosslinking of putative precursor proteins to form the insoluble cell envelope. There are three enzymes active in the epidermis, transglutaminases 1, 2 and 3. We have characterized the full-length cDNA and genes encoding transglutaminases 1 and 3, and mapped their chromosome locations. Full-length and deletion constructs of the transglutaminase 1 and 3 enzymes have been expressed in bacteria and characterized. A new antibody has been prepared for the transglutaminase 1 enzyme and used to study the expression in the epidermis.



## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Genetic Studies of Hereditary Skin Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	S Bale	Acting Chief	GSS, LSB, NIAMS
Others:	A Wright	Biologist	GSS, LSB, NIAMS
	J Digiovanna	Sen Staff Fellow	CDIU, IRP, NIAMS
	V Kimonis	Visiting Fellow	GSS, LSB, NIAMS
	J Lin	IRTA Fellow	GSS, LSB, NIAMS
	J Compton	Sen Staff Fellow	GSS, LSB, NIAMS
	S Doyle	Research Nurse	GSS, LSB, NIAMS
	G Richard	Dermatologist	Erfurt Univ, Germany
	C Chipev	Visiting Assoc	LSB, NIAMS
	L Russel	Sen NRC Fellow	
	J-M Yang	Visiting Fellow	LSB, NIAMS
	P Steinert	Branch Chief	LSB, NIAMS

## COOPERATING UNITS (if any)

OW McBride, Section Chief, LB, DCBDC, NCI; A Goldstein, Sr. Staff Fellow, EEB, DCE, NCI; N Hashem, Professor, Ain-Shams Medical Genetics Center, Cairo, Egypt; C Mazzanti, Istituto Dermaopatico dell'Immacolata, Rome, Italy

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Genetic Studies Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

4.1

## PROFESSIONAL:

2.5

## OTHER:

1.6

## CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the genetic basis of a variety of skin disorders, including six disorders of cornification (epidermolytic hyperkeratosis, lamellar ichthyosis and congenital ichthyosiform erythroderma, Darier's disease, Hailey-Hailey disease, and erythrokeratoderma variabilis) and one disorder predisposing to skin cancer (basal cell nevus syndrome). Patients and their families are recruited for study. They travel to the NIH clinics (or rarely we travel to them) to enable our research group to perform detailed family and medical histories, skin examinations, skin biopsies, phlebotomy, and clinical photography. DNA is extracted from patient's blood or buccal cells. Skin samples are used to confirm diagnoses and to investigate ultrastructural abnormalities specific to each disease. Clinical heterogeneity (different clinical appearances of the "same" disease) is investigated using the information collected. DNA-based polymorphisms (i.e. RFLPs, PCR) are used for linkage studies to determine the chromosomal location of the skin disease locus. Identification of the disease-causing gene is made by searching for mutations in candidate genes in the mapped regions. Genotype-phenotype correlations are drawn based on the clinical presentation and the specific gene mutation.



## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Development of New Statistical Methods for Genetic Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sherri J. Bale, Ph.D. Acting Chief LSB, NIAMS

Others: Jing-Ping Lin, Ph.D. IRTA Fellow LSB, NIAMS

## COOPERATING UNITS (if any)

CI Amos, M.D., Anderson Medical Center, Houston, TX

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Genetic Studies Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

1.1

## PROFESSIONAL:

1.1

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We continue to provide consultation and support (analytic, computer software, computation) for various investigators, both intramural and extramural, who are interested in assessing the genetic component of diseases, and we develop new methods of analysis where needed to fulfill the needs of these investigators. We have again worked this fiscal year with several scientists, both intramural (NIAMS and other NIH institutes) and extramural (both NIH and outside institutions) to assist in the design and execution of studies to 1) assess familial aggregation of disease, 2) investigate linkage relationships between disease and genetic markers, 3) assess the relative risks of various environmental components to the development of disease, and 4) provide software support for genetic analysis programs.



## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Consultation for Genetic Analyses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-P.I.: SJ Bale Acting Chief GSS,LSB,NIAMS

Co-P.I.: C Amos Staff Fellow GSS,LSB,NIAMS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Genetic Studies Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

0.6

## PROFESSIONAL:

0.6

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We provide consultation and support (analytic, computer software, computation) for various investigators, both intramural and extramural, who are interested in assessing the genetic component of diseases. We have worked this fiscal year with several scientists, both intramural (NIAMS and other NIH institutes) and extramural (both NIH and outside institutions) to assist in the design and execution of studies to 1) assess familial aggregation of disease, 2) investigate linkage relationships between disease and genetic markers, 3) assess the relative risks of various environmental components to the development of disease, and 4) provide software support for genetic analysis programs.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AR 41093-03 LSB

## PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression, Structure and Function of Trichohyalin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert	Chief	LSB,NIAMS
Others:	Seung Chul Lee	Visiting Fellow	LSB,NIAMS
	Edit Tarcza	Visiting Fellow	LSB,NIAMS
	Lyuben Marekov	Visiting Scientist	LSB,NIAMS
	Nedialka Markova	Visiting Scientist	LSB,NIAMS

## COOPERATING UNITS (If any)

Soo-II Chung, Senior Investigator, LCDO\NIDR; Alesando Finazzi-Agro, Genaro Melino, Giampiero Mei, Department of Experimental Medicine, University Tor Vegata, Rome, Italy

## LAB/BRANCH

Laboratory of Skin Biology

## SECTION

Molecular Biology of Keratinization Section

## INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

1.5

## PROFESSIONAL:

1.5

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Trichohyalin is a major differentiation product of the inner root sheath cells of hair follicle, of the medulla of the hair fiber, and is expressed in minor amounts in the epidermis. It is thought to function as a keratin intermediate filament associated protein in these tissues. In addition, trichohyalin is a substrate for transglutaminases, and for the enzyme peptidyl arginine deiminase can convert up to about 40% of the arginines of this protein into citrullines, whereupon the protein becomes insoluble. Structural studies of the protein using circular dichroism and fluorescence spectroscopy, before and after crosslinking or arginine modification, are in progress to study the structure of the protein. An analysis of the regulatory sequences which control the expression of the trichohyalin gene have shown that the first 100 bp above the transcription start point are almost identical to the profilaggrin gene, which means that most of the sequences required for epithelial-specific expression are located on proximal promoter elements. We have made constructs containing upstream sequences coupled to a beta-galactosidase reporter to explore the expression of this gene in transgenic mice.





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